factor at 2000 units mL-1 and bFGF 4 ng/ml d) Culturing said embryos in said medium until day 12 e) Fixing and analyzing by FISH said embryonic cell lines Identifying and isolating disomic cell lines within said embryonic cell lines wherein disomic cell lines are produced.

Rewrite as
Claim 3) I claim the method of producing disomic cell lines comprising the steps of:

a) culturing trisomic embryos onto mouse feeder cells consisting of mouse embryonic fibroblast cells (ATCC-STO) said mouse embryonic fibroblast cells having been previously mitotically inactivated by [[mitocimin]] mitomycin C in gelatin-tissue culture dishes, and
b) maintaining said mouse feeder cells using Dulbecco's Modified Eagle Medium (DMEM) without sodium pyruvate, glucose 4500 mgL-1 supplemented with 20% fetal bovine serum, 0.1 mM -mercaptoethanol, 1% non-essential amino acids, 1 mM L-glutamine, 50 units ml L-1 penicillin, and
c) supplementing said medium with human recombinant Leukemia Inhibitory factor at 2000 units mL-1 and bFGF 4 ng/ml
d) Culturing said embryos in said medium until day 12, and
e) fixing and analyzing by FISH said embryonic cell lines, and
identifying and isolating disomic cell lines within said trisomic embryos wherein disomic cell lines are produced.

I have added "I claim the" and deleted a capital "A" as you have suggested re: MPEP 608.01(m) and in your office action of 11/23/2005 as it is one method.
I have added "trisomic embryos" and deleted "embryonic cell lines" which you suggested in your office action of 3/23/2007 as it did not have an antecedent basis. "trisomic embryos" have an antecedent basis in Claim 1. I have corrected the typo on "mitomicin" to "mitomycin".

4. A method of claim 3 wherein stem cell lines are isolated from said disomic cell lines.
Rewrite as:
Claim 4) I claim a method of claim 7 wherein stem cell lines are isolated from said disomic cell lines.

I have added "I claim a" and deleted a capital "A" as you have suggested re: MPEP 608.01(m) and deleted claim 3 and have added claim 7 as suggested in your non-final office action of 11/23/2005

I attach a separate sheet of paper with a "clean" copy of claims 1 -4, which is now renumbered as Claims 5, 6, 7, 8 and Claims 1 through 4 have been cancelled. I assume the Technical Section will then renumber these claims if allowed as 1 through 4 at the end of this examination process.

Re: Oath and Declaration, You have previously accepted the new signed Oath and Declaration with the Citizenship corrected from the ambiguous "American" as you pointed out to "USA".

Application Number 10/625,100
Filing Date 07-22-2003
Application Type: Utility
Examiner: Ton, Thaian N
Group Art Unit 1632
Class/Sub-Class 436/366
Publication No"US2005-0019907 A1
Publication Date 01-27-2005
Inventor Santiago Munne
Address of inventor: 55 Lakeview Avenue, Shorthills, NJ 07078, tel 646-207-2897
Title : Obtaining normal disomic stem cells from chromosomally abnormal embryos

Copy of Claims With Mark Up: Cancel Claims 1 to 4 and replace with Claims 5,6,7,8

Claim 5 (Currently amended) I claim a [[A]] disomic cell line derived from trisomic embryos.

Claim 6 (Currently amended) I claim a [[A]] stem cell line derived from said disomic cell line [[s]] of claim 5.

Claim 7 (Currently amended) I claim the [[A]] method of producing disomic cell lines comprising [[consisting of]] the steps of:
a) culturing trisomic embryos onto mouse feeder cells consisting of mouse embryonic fibroblast cells (ATCC-STO) said mouse embryonic fibroblast cells having been previously mitotically inactivated by mitomycin [[mitocimin]] C in gelatin-tissue culture dishes; and,
b) maintaining said mouse feeder cells using Dulbecco's Modified Eagle Medium (DMEM) without sodium pyruvate, glucose 4500 mgL-1 supplemented with 20% fetal bovine serum, 0.1 mM -mercaptoethanol, 1% non-essential amino acids, 1 mM L-glutamine, 50 units ml L-1 penicillin; and,
c) supplementing said medium with human recombinant Leukemia Inhibitory factor at 2000 units mL-1 and bFGF 4 ng/ml; and,
d) culturing said embryos in said medium until day 12; and,
e) fixing and analyzing by FISH said embryonic cell lines, and
identifying and isolating disomic cell lines within said trisomic embryos [[embryonic cell lines]] wherein disomic cell lines are produced.

Claim 8) (Currently amended) I claim a method of claim 7 [[3]] wherein stem cell lines are isolated from said disomic cell lines.

CLAIMS

Claim 5 (Currently amended) I claim a disomic cell line derived from trisomic embryos.

Claim 6 (Currently amended) I claim a stem cell line derived from said disomic cell line of claim 5.

Claim 7 (Currently amended) I claim the method of producing disomic cell lines comprising the steps of:

a) culturing trisomic embryos onto mouse feeder cells consisting of mouse embryonic fibroblast cells (ATCC-STO) said mouse embryonic fibroblast cells having been previously mitotically inactivated by mitomycin [[mitocimin]] C in gelatin-tissue culture dishes; and,

b) maintaining said mouse feeder cells using Dulbecco's Modified Eagle Medium (DMEM) without sodium pyruvate, glucose 4500 mgL-1 supplemented with 20% fetal bovine serum, 0.1 mM -mercaptoethanol, 1% non-essential amino acids, 1 mM L-glutamine, 50 units ml L-1 penicillin; and,

c) supplementing said medium with human recombinant Leukemia Inhibitory factor at 2000 units mL-1 and bFGF 4 ng/ml; and,

d) culturing said embryos in said medium until day 12; and,

e) fixing and analyzing by FISH said embryonic cell lines, and

identifying and isolating disomic cell lines within said trisomic embryos wherein disomic cell lines are produced.

Claim 8) (Currently amended) I claim a method of claim 7 wherein stem cell lines are isolated from said disomic cell lines.

Claim Rejection -35 USC 112, Claims 7 and 9

Examiner suggests that the specification "while being enabling for methods of producing disomic human embryonic cell lines" is not enabling for making "stem cell lines". The specification incorporates several articles and patents that teach to "any person skilled in the art" of making stem cells. Patent specification need only describe and show possession of the invention in its totality and not teach (Hybridtech v Monoclonal antibody inc 231U.S.P.Q.81 (federal circuit 1986)), herself, states that Thompson patents and reference's teach how to make stem cells from "normal" non trisomically derived disomic embryonic cells. "Thomson et al ...teach the specific art-recognized" What Thompson did not teach, nor anticipate, is that trisomic cell lines can revert to "normal" disomic cell lines. In re Edwards, 568 F 2d at 1351, 196 U.S.P.Q.2d at 468 the Court held that the detailed disclosure of the process and possible reactants were sufficient to provide a "written description" of one of the possible products of that reaction. And, In re Ruschig 54 CCPA 1551, 154 USPQ 118 (CCPA 1967) that a sufficient disclosure is "one that marks a trial through the woods by supplying blaze marks on the trees". We believe we have marked a clear trail to making stem cells from trisomically derived disomic embryonic cell lines.

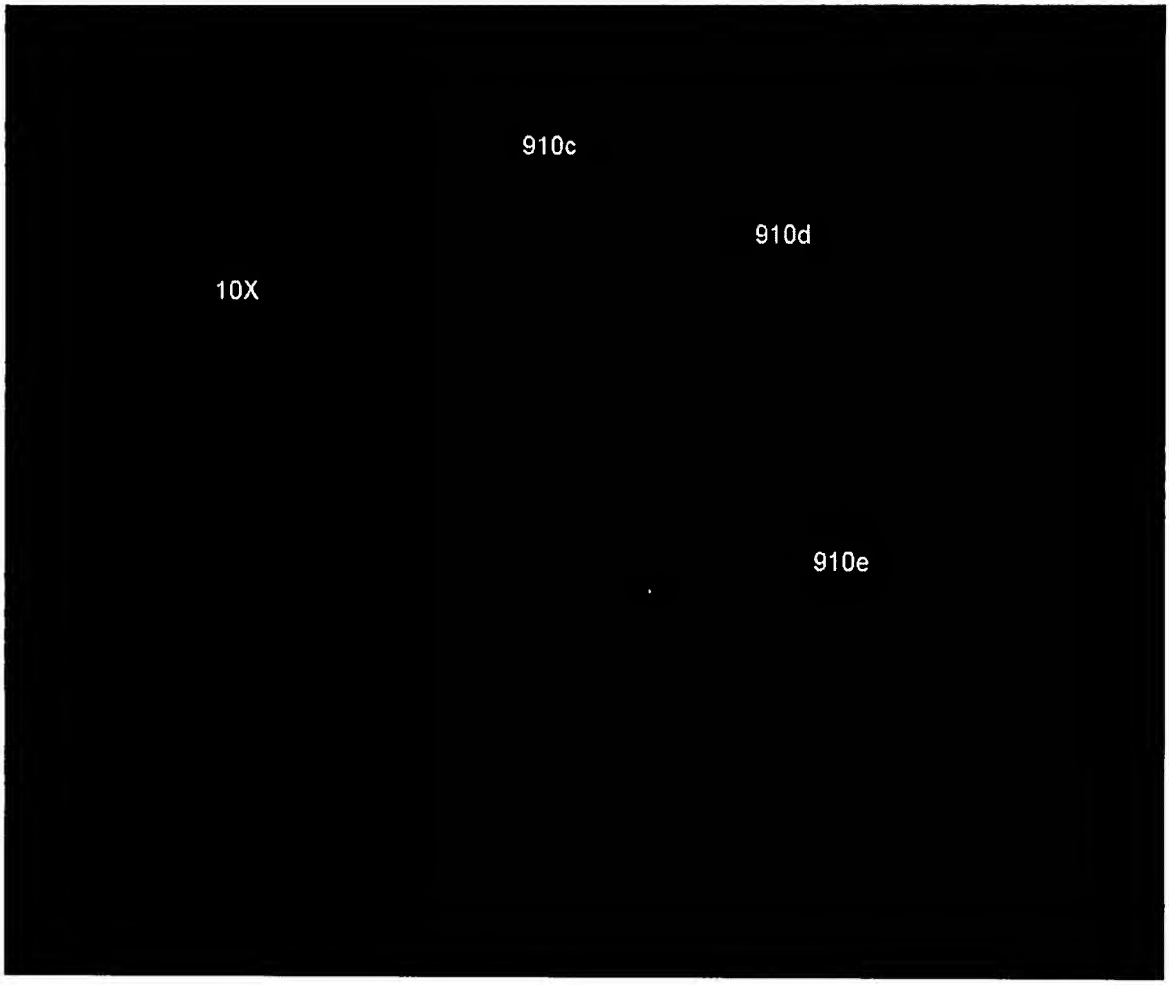
Examiner brings in a new ground of rejection which is the Wanda factors. We quote from sections of *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (reversing the PTO's determination that claims directed to methods for detection of hepatitis B surface antigens did not satisfy the enablement requirement). In Wands, the Court held that the specification was enabling with respect to the claims at issue and found that "there was considerable direction and guidance" in the specification; there was "a high level of skill in the art at the time the application was filed;" and "all of the methods needed to practice the invention were well known." After considering all the factors related to the enablement issue, the court concluded that "it would not require undue experimentation to obtain antibodies needed to practice the claimed invention." This clearly falls under the Wanda factors, but the Examiner's interpretation fails to note that obtaining an antibody for a stem cell such as TRA-1-60, SSEA-1, SSEA-3, SSEA-4, TRA 1-81, OCT 4 or alkaline phosphatase does not require undue experimentation. In

fact, these antibodies are commercially available and testing these disomic with said antibodies also does not require undue experimentation. Therefore, under Wanda testing for epitopes to structurally define the trisomically derived disomic cells which are stem cells does not require undue experimentation. The claim breadth, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, the amount of experimentation are all satisfied by simply identifying even one stem cell epitope on one cell of a population of trisomically derived disomic cells. This can be done by using one antibody such as TRA-1-60 to see if any cells in the population of trisomically derived disomics bind said antibody or a mimic thereof.

In the micrograph below, we show just such a population of cells binding a TRA-1-60 rhodamine-labeled peptide mimic of TRA-1-60 binding antibody. This did not require undue experimentation in that it only required adding said rhodamine peptide mimic of TRA-1-60 to the slide and viewing said population under a fluorescence microscope. The very bright cells are the trisomically derived disomic stem cells binding the TRA-1-60 ligand mimic. The dark cells are the non-binding differentiating cells. Anyone skilled in the art could repeat this simple procedure. It is just a matter of adding 1 microliter of stain to the sample and viewing same.

I have attached a notarized Rule 1.132 affidavit from Sasha Sadowy attesting to her ability to reproduce and enable the procedures and methods outlined in the application and to achieve similar if not identical results. Sasha Sadowy is a licensed embryologist and is of "ordinary-skill in the arts" in making and identifying stem cell lines.

This evidence should address your new 35 USC 112 para 1 enablement objections to claims 7 and 8.



In addition, I can testify, not as a "counsel" as you suggested, but as a *pro se* inventor and scientist, that I have assigned other colleagues who are skilled in the art in making stem cells from these cell lines with only the specification and references detailed in this patent application and they have successfully isolated stem cells (RG44, RG56, RG92, RG93, RG94, RGK230) that have the characteristic epitopes of stem cells (SSEA-1, SSEA-3, SSEA-4, TRA 1-60, TRA 1-81, OCT 4, alkaline phosphatase). In *Re Shulze*, does not address an inventor giving an affidavit on a scientific matter where the inventor is also the *pro se* applicant. It is understandable that a lawyer may not have the experience to validate the reproducibility of a research protocol.

We believe that this micrograph provides the "nexus" between the disomic cells, characterized by karyotyping and FISH, and the derived stem cell population characterized by the TRA-1-60 epitope, using a rhodamine labeled ligand.

The specification therefore does describe how to make and use the invention of making a disomic cell line from a trisomic embryos and identifying with a simple viable stem cell specific staining procedure the stem cells within the disomic cell population.

Experiments are in progress to take the isolated stem cells so identified and injecting them into mice to create the classical functional end point assay of stem cell differentiation into a teratoma containing all three primary germ layers.

Examiner has previously acknowledged that the specification provides guidance to show that embryonic cells can be produced, but examiner failed to acknowledge that the inherent characteristic of embryonic cells is that by definition embryonic cells have differentiation potential of pluripotent cells. Pluripontent differentiation is an intrinsic *a posteriori* function for an *a priori* defined embryonic cell.
Examiner herself almost alludes to this in her anticipation argument of Claims 4 and 5 claiming that disomic cells are the equivalent to the disomic cell lines of Thompson's definition of stem cells.

Claim Rejection of 35 USC102
Examiner rejects Claims 5 and 6 as being anticipated by Thomson because examiner believes that all embryonic cell lines created by Thomson are disomic. Examiner makes the erroneous assumption that all disomic cell lines irrespective of origin are identical in structure and function without presenting any evidence for this assumption. This assumption is being presently tested. For example, disomic cell lines derived from trisomic can be quantitatively different from disomic cell lines derived from non-trisomic origins. Disomic cell lines derived from trisomic parentals have the possibility of being uniparental. Neither Thomson nor Shamblatt's disomic cell lines can be uniparental. Therefore, their anticipation has little to do with these disomic cell lines.

What does uniparental mean? How do they arise? and how can it be tested?
Firstly, a uniparental disomic cell line differs in having two copies of one chromosome type, i.e. chromosome 18 (the one originally trisomic, i.e. trisomy 18) from one parent

and none from the other, while stem cell lines derived from euploid embryos produce disomic cell lines with one chromosome from each parent. The specification has outlined various methods of their derivation. Therefore, while they have a full disomic genetic complement, they also have each gene expressed twice on a given chromosome. This is not the disomics which Thomson and Shamblatt describe. Their disomics have one chromosome set from each parent and each allele must therefore be expressed in competition with its corresponding partner. Recessive genes can be suppressed by dominant genes in a biparental. This is not the case in a uniparental. All genes for the chromosome affected are expressed equally. Recessives and dominant genes are expressed both expressed. There are no heterozygotic disomic cells for that chromosome when they are uniparental.

Therefore, the stem cells derived from uniparental disomic cells derived from trisomics will have two identical allelic copies of each gene for the chromosome affected. This is not the same disomic cell type anticipate by Thomson and the normal disomic karyotype anticipated by Shamblatt. If there must be a characterization of this disomic cell type, it can be called a uniparental disomic cell or UPD.

We are presently running the experiments to demonstrate the uniparental nature of these disomics derived from trisomics. PCR experiments are being done on parents of embryo donors to identify allele's characteristic of each parent. Disomic cells derived from trisomic cells of these embryos are being analyzed for these alleles. The identification of UDP's will rest on identifying cells which contain two copies of the same allele in the affected chromosome, which are normally different in the parents. This is a prophetic example of these UDPs. In addition, Shamblatt has isolated "normal" disomic human stem cells from 5 to 9 week gonadal ridge cells. 5 to 9 week cell types have differentiated and would require a de-differentiation procedure to become totipotent. Shamblatt's cells are heterozygotic containing alleles from both parents.

These UDP's differ genotypically in character from anything described by Thomson and Shamblatt, they are completely homozygotic for the chromosome affected; and, therefore, may have stem cell epitopic markers in twice the number, making them easier

to identify, detect and isolate. This will depend if the uniparental chromosome has genes which code for stem cell epitopes for which antibodies or binding ligands exist.
The references presented by Examiner and anticipation requires all the elements of the claim a priori and not just a mix of elements and a hind sight combination *a posteriori* (Hybridtech v Monoclonal Antibody inc 231U.S.P.Q.81 (federal circuit 1986)). The uniparental element and trisomic origin element of the claim 4 and 5 are not mentioned or conceived in either Thomson or Shamblatt.

Claim 7 and 8 is not anticipated by Thomson for the same reason that Claim 4 and 5 are not. The method describe are methods to isolate UPD, *inter alia*. This method could not have been conceived by Thomson as the underlying product's genotype, nor its duplicate allelic expression for the affected chromosome, was not anticipated by Thomson. Therefore, we submit that Claims 4, 5, 6, 7 are properly described, the inventor having complete possession of the invention; and, that the specification and claims allow any practitioner in the art to make trisomically derived uniparental disomic cells, *inter alia* and uniparental stem cells derived there from. That neither Thomson nor Shamblott, separately, nor put together, *a posteriori*, anticipate all elements of the claim, nor even the concept of trisomically derived disomic nor uniparental disomic cell lines.

We plead that examiner accept the claims and consider our other arguments, experimental results as, prophetic experiments and disputations. In addition, we suggest that examiner under Rule MPEP 707.07(j), if the Examiner finds patentable subject mater disclosed, but feels that Inventor's present claims are not entirely suitable, the Examiner draft one or more allowable claims for the applicant.

Sincerely yours,

Santiago Munne, Pro Se Inventor